Accelerated Solvent Extraction Techniques for In-Line Selective Removal of Interferences

Introduction

Interferences may be extracted along with desired analytes during an extraction process. These unwanted co-extractables may interfere with analyte detection or decrease instrument performance. Traditionally, chromatographic techniques such as gel-permeation chromatography (GPC) or a glass column packed with specific adsorbents are used to purify sample extracts prior to separation and analysis. Recent advances using accelerated solvent extraction systems, as described in several publications,[1,3–10,12–17] include procedures for selective removal of interferences during sample extraction, thus combining extraction and purification into a single step.

This application note summarizes seven accelerated solvent extraction procedures developed to remove co-extractable material from various matrices: procedures to selectively extract polar compounds from lipid-rich samples and to fractionate lipids from biological samples.

This note is intended to serve as a guide to develop accelerated solvent extraction methods. For more information, please refer to the original publication cited with each method described below, or contact us.

Selective Extraction of Nonpolar Compounds

In an effort to eliminate post-extraction cleanup steps, we and others have researched the addition of various adsorbents to the extraction cell. For many sample types, this approach has proven successful in producing clean extracts that are ready for direct analysis. For example, nonpolar lipids are often co-extracted from fish tissue. Adding alumina (aluminum oxide, Al_2O_3, acidic, activated by placing in a drying oven at 350 °C for 15 h) to the extraction cell before adding the sample or sample mixture has been shown to prevent the extraction of unwanted lipids. Mixing the sample with C18 resin (1:2) has been shown to retain organic contaminants. (C18 bonded silica, 35–70 µm diameter, and porosity of 60 Å, from Alltech has been used, but similar materials from other vendors can be used.)

When in-cell cleanup is performed during accelerated solvent extraction solvent choice impacts the retention of unwanted components. For example, a mixture of hexane/acetone (1:1) is a common solvent for extracting organochlorine pesticides from animal tissues. After extraction, a cleanup step is usually required to remove co-extracted lipids. Adding alumina to an accelerated solvent extraction cell and extracting with hexane can prevent the extraction of interferences. However, if hexane/acetone (1:1) is used as the extraction solvent, almost no lipid material will be retained on the alumina. Table 1 lists two types of fat retainers and the ratio of each required to retain fat when using nonpolar solvents. Table 2 lists common adsorbents that are used for selective extraction of compounds using accelerated solvent extraction systems.

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Selected Techniques for In-Line Cleanup of Various Nonpolar Compounds

Selective Extraction of PCBs from Fish Meal Samples Using Acid-Impregnated Silica Gel[5,6]

Adsorbents and Dispersants

Silica gel
Thermo Scientific Dionex ASE Prep DE (P/N 062819)
Fisher Scientific sodium sulfate*
Fisher Scientific Ottawa sand

* Sodium sulfate can be used only when using hexane or heptane as an extraction solvent. If using any other solvent, it is important to use Dionex ASE™ Prep DE.

Table 1. Ratio of Fat Retainer per Gram of Lipid Using Nonpolar Solvents

Fat Retainer	Ratio of Fat Retainer/Lipid (g/g)
Alumina	20–25
Acid-impregnated silica gel	45.5

The nonpolar solvents that give the best results are hexane and heptane. Increased extraction temperatures and increased solvent polarity will decrease the fat retainer's effectiveness.

Table 2. List of Adsorbents and Uses

Adsorbent*	Uses
Carbon	Removes organics and nonpolar compounds (used in dioxin analysis)[1]
Copper	Removes sulfur[2]
Ion-exchange resins**	Removes organics, ionic interferences for IC and IC/MS analysis[3]
CS18 resin	Removes organics, polar compounds, lipids, colors[4]
Acid-impregnated silica gel**	Removes lipids[5,6]
Alumina**	Removes nonpolar lipids, colors[7,8,9]
Florisil® (U.S. Silica Co., Frederick, MD, USA)	Removes nonpolar lipids[10,11]
Silica gel	Removes nonpolar lipids[12]

** Note: We do not recommend using sodium sulfate and magnesium sulfate as in-cell adsorbent agents with polar solvents because of potential clogging problems. Use of sodium or magnesium sulfate can also contribute to the failure of key accelerated solvent extraction instrument components such as the static valve.

**These selected techniques are detailed below.

Preparation of 40% Sulfuric Acid-Impregnated Silica Gel

Heat 600 g of silica gel at 200 °C for at least 12 h. Allow to cool. Add 400 g of sulfuric acid to the cooled silica gel. Mix together in a tumbling mixer for 4 h.

Extraction Conditions

Extraction Solvent:	n-Hexane
Pressure:	1500 psi
Temperature:	100 °C
Static Time:	5 min
Static Cycles:	2
Flush:	60%
Purge:	90 s
Cell Size:	33 mL

Extraction Cell Preparation

Prior to extraction, prepare a 33-mL cell as shown in Figure 1. Begin by inserting two cellulose filters at the outlet end of the accelerated solvent extraction cell. Add a layer of untreated silica gel then a layer of the acid-impregnated silica gel. Place a cellulose filter on top of the acid-impregnated silica gel then add the sample, as prepared below. Fill any remaining cell volume with Ottawa sand. (Placing a cellulose filter on top of the Ottawa sand is optional.)

Sample Preparation

Grind 2 g of fish meal sample with 2 g of sodium sulfate and 2 g Ottawa sand using a mortar and pestle. Add this sample mixture to the cell as shown in Figure 1. Place the extraction cell, containing the sample, on the accelerated solvent extraction system and extract with the method conditions listed above. Concentrate the extracts to approximately 1 mL then dilute to a final volume of 2 mL with hexane. Analyze using EPA Method 8082.

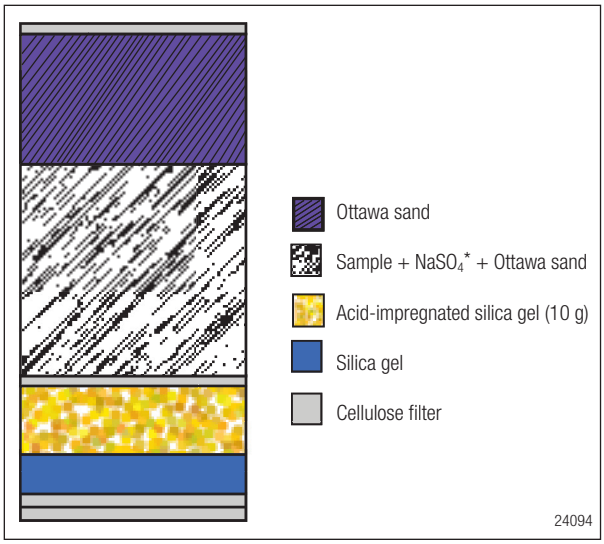

Figure 1. Preparation of the accelerated solvent extraction cell for the selective extraction of PCBs from fish meal.

Extraction Cell Preparation

Prior to extraction, prepare the 33-mL cell by placing one cellulose filter at the outlet. Place the activated alumina on top of the filter, as shown in Figure 2.

Sample Preparation

Grind 3 g of fish tissue with 15 g of sodium sulfate in a mortar and pestle. Transfer this mixture to the 33-mL extraction cell as shown in Figure 2. Rinse the mortar and pestle with 2 mL of hexane and add to the extraction cell. Place the extraction cell containing the sample on the accelerated solvent extraction system and extract with the method conditions listed above. Analyze extracts using EPA Method 8082.

The amount of alumina required to retain the fat in the sample depends on the fat content of the fish tissue and the mass of the tissue sample extracted (Table 1).

Selective Extraction of PCBs from Fish Tissue Using Alumina[7]

Adsorbents and Dispersants

Dionex ASE Prep DE (P/N 062819)
Fisher Scientific sodium sulfate*
Alumina 60–325 mesh, Brockman activity I, Fisher Scientific acidic

*Sodium sulfate can be used only when using hexane or heptane as an extraction solvent. If using any other solvent, it is important to use Dionex ASE Prep DE.

Preparation of Activated Alumina

Heat the alumina at 350 °C for 15 h.

Extraction Conditions

Extraction Solvent:	n-Hexane
Pressure:	1500 psi
Temperature:	100 °C
Static Time:	5 min
Static Cycles:	2
Flush:	60%
Purge:	120 s
Cell Size:	33 mL

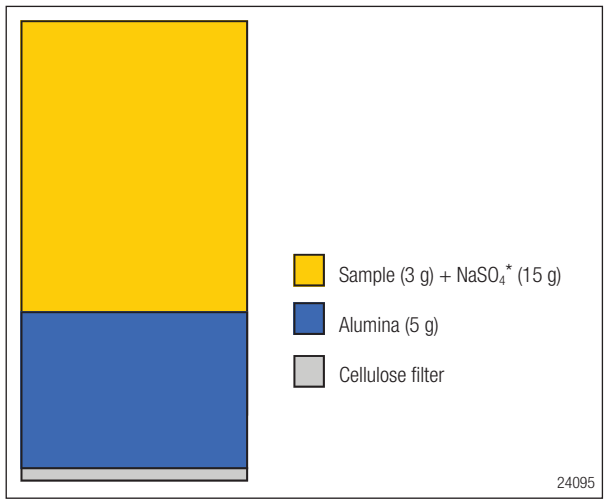

Figure 2. Preparation of the accelerated solvent extraction cell for the selective extraction of PCBs from fish tissue using alumina.

Selective Extraction of Polar Compounds from Polar Matrices

Selective Extraction of Perchlorate from Vegetation Using Ion-Exchange Resins[3]

Ion-Exchange Resins and Adsorbents

Thermo Scientific Dionex OnGuard II Sample Pretreatment Cartridges
Ag (P/N 057089)
Ba (P/N 057093)
H (P/N 057085)
RP (P/N 057083)
Dionex ASE Prep DE (P/N 062819)
Fisher Scientific basic alumina

Extraction Conditions

Extraction Solvent:	HPLC-grade water
Pressure:	1500 psi
Temperature:	80 °C
Static Time:	5 min
Static Cycles:	3
Flush:	30%
Purge:	120 to 240 s
Cell Sizes:	33 mL or 100 mL

Extraction Cell Preparation

Prior to extraction, prepare the 100-mL cell by inserting two glass-fiber filters in the outlet end of the accelerated solvent extraction cell. Cut the end off each Dionex OnGuard™ resin cartridge and store the resins in separate beakers until needed. Add the OnGuard resins, the alumina, and the glass-fiber filters as shown in Figure 3. Fill any remaining cell volume with Dionex ASE Prep DE. If using 33-mL cells, prepare in the same manner with proportionally less of each adsorbent. To ensure clean adsorbents and resins, extract each prepared cell with the accelerated solvent extraction method conditions listed above before adding the sample.

Sample Preparation

Remove 10 g of "clean" Dionex ASE Prep DE from the extraction cell and grind together with 5 g of vegetation sample. Add the sample mixture back to the extraction cell. Place the extraction cell containing the sample on the accelerated solvent extraction system and extract with the method conditions listed above. Adjust the final volume of each extract to either 40 mL (if a 33-mL accelerated solvent extraction system cell was used) or 100 mL (if a 100-mL cell was used).

Selective Extraction of Corticosteroids from Bovine Liver[13]

This method describes a fractionation extraction to remove nonpolar triglycerides (defatting step) followed by a second extraction of the sample to recover analytes of interest.

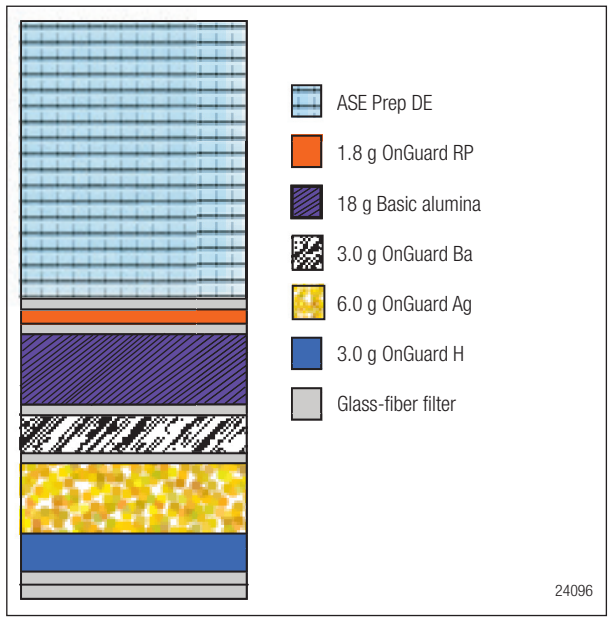

Figure 3. Preparation of the accelerated solvent extraction cell for the selective extraction of perchlorate from vegetation.

Extraction Conditions

	Defatting	Extraction
Solvent:	n-Hexane	Hexane/ethyl acetate (1:1, v/v)
Temperature:	60 °C	50 °C
Pressure:	1500 psi	1500 psi
Static Time:	5 min	5 min
Flush:	100%	60%
Purge:	100 s	100 s
Static Cycles:	3	1
Cell Size:	22 mL	22 mL

Sample Preparation

Mix 5 g of liver tissue sample with 7.5 g of Dionex ASE Prep DE and load into a 22-mL extraction cell containing a cellulose filter at the outlet end. First extract with the defatting conditions listed above and re-extract the sample using the analyte extraction conditions. Evaporate second extract to dryness and dissolve in 4 mL of absolute ethanol. Centrifuge at 4000 rpm. Remove the ethanol layer and evaporate to dryness. Dissolve residue in 500 µL of methanol. Analyze by LC/MS.

Selective Extraction of Sulfonamide from Animal Tissue[4,14]

This method describes the use of C18 resin to remove most of the nonpolar lipids from animal tissue samples during extraction. The remaining co-extracted polar lipids are precipitated from the extract by cooling and separated by centrifugation. The resulting supernatant is analyzed using HPLC/MS/MS.

Extraction Conditions

Extraction Solvent:	HPLC-grade water
Pressure:	1500 psi
Temperature:	160 °C
Static Time:	5 min
Static Cycles:	1
Flush:	60%
Purge:	60 s
Cell Size:	11 mL

Sample Preparation

Homogenize the meat samples using any standard tissue homogenizer. This should be done with HPLC-grade water added to the sample. Set the homogenizer to 5,000 rpm. Once the homogenizer reaches 5,000 rpm, increase speed to 25,000 rpm and hold for 15 min. Evaporate excess water. Weigh out approximately 2 g of homogenized tissue and mix with 4 g of C18 until the entire mixture is of a uniform consistency. Transfer this mixture to an 11-mL accelerated solvent extraction cell containing a glass-fiber filter.

Place the cell onto the Thermo Scientific Dionex ASE 200 accelerated solvent extraction system. Program the Dionex ASE™ 200 accelerated solvent extraction system with the conditions above and start the extraction. After the extraction, place the sample vials in a freezer (–18 °C) for one hour to precipitate the lipids.[14] Remove the extracts from the freezer and centrifuge for 5 min at 10,000 rpm. Analyze the supernatant using HPLC/MS/MS as described in reference 4.

Extraction of Acrylamide from Cocoa and Coffee Samples Using In-Cell Cleanup[15]

The addition of Florisil to the extraction cell greatly simplifies the analysis of acrylamide from complex food samples such as cocoa and coffee. This fact is illustrated in Figure 4. Coffee samples, each with different amounts of Florisil added to the accelerated solvent extraction cell, were extracted and loaded into Isolute® (Biotage AB, Uppsala, Sweden) Multimode SPE cartridges. Figure 4 clearly shows a decrease in the amount of visible co-extractable material as the amount of Florisil in the accelerated solvent extraction cell increased. The authors report that 6 g of Florisil is sufficient to produce a clear extract. For complete details of the accelerated solvent extraction conditions and sample preparation see Application Note 358.[15]

Lipid Fractionation of Biological Samples[16,17]

The following accelerated solvent extraction method was developed to fractionate nonpolar neutral lipids and polar phospholipids from biological samples. The fractionation takes place in a single accelerated solvent extraction cell containing a specific adsorbent that retains polar phospholipids while nonpolar lipids are extracted. Once the nonpolar lipids are extracted, the sample is re-extracted with a more polar solvent, which removes the polar phospholipids. Each extract is directed into a separate collection vial, allowing fractionation of the two classes of lipids. This fractionation is feasible due to the flow-through design of the accelerated solvent extraction system. The solvent controller allows the sequential use of different extraction solvents. For example, a single accelerated solvent extraction schedule can be used to automate an extraction with hexane/acetone (9:1) at 50 °C to remove triglycerides, followed by an extraction with chloroform/methanol (1:4) to isolate polar lipids such as phospholipids or hydroxyl-fatty acids. Additional details about this method can be found in references 16 and 17.

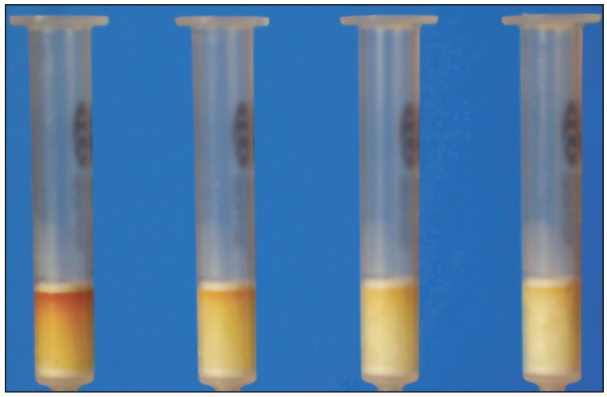

Figure 4. Residual co-extractives after the accelerated solvent extraction of a coffee sample trapped on an Isolute Multimode SPE cartridge, the accelerated solvent extraction cells containing from 0 to 6 g Florisil. From left to right: 1) no Florisil in the accelerated solvent extraction cell, 2) 2 g Florisil in the accelerated solvent extraction cell, 3) 4 g Florisil in the accelerated solvent extraction cell, and 4) 6 g Florisil in the accelerated solvent extraction cell.

Conclusion

The flow-through design of the accelerated solvent extraction system allows the system to perform extractions in combination with in-line cleanup. Eliminating post-extraction cleanup steps reduces solvent usage and waste, and saves the operator time and labor. This makes the accelerated solvent extraction system a powerful tool for total sample preparation.

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